**Effect of Bio-stimulant (Plant Probiotics) on Growth, Yield and Microbial Activity of Chickpea (*Cicer arietinum* L.) Grown in vertisol of Chhattisgarh**


## Abstract

A field experiment was conducted to find out the effect of biostimulant (plant probiotics) on growth, yield, and the microbial activity of Chickpea (*Cicer arietinum* L.) grown in vertisol during the *rabi* season (December–April) of 2022–23 at the Instructional Cum Research Farm, College of Agriculture, IGKV, Raipur, Chhattisgarh, India. The RVG-202 variety of chickpea was used for the experiment, which was spaced in 45×10 cm. The experiment consists of seven treatments, viz., $T_1$ (Control (without RDF)), $T_2$ (100% RDF), $T_3$ (1 L biostimulant + 200 L water acre$^{-1}$), $T_4$ (1 L biostimulant + 100 L water acre$^{-1}$), $T_5$ (1 L biostimulant + 300 L water acre$^{-1}$), $T_6$ (1 L biostimulant + 200 L water acre$^{-1}$ + 100% RDF) and $T_7$ (1 L Biostimulant + 200 L water acre$^{-1}$ + 50% RDF) and was laid out in a randomized block design (RBD) with three replications. Data regarding the Plant height, shoot dry weight, nodule number, nodule dry weight, pod number, grain yield and stover yield, microbial population, NPK content in grain and stover were observed. The experimental result revealed that the application of biostimulant with a combination of 50% RDF was found to have a significant impact on the Plant height, shoot dry weight , nodule number, nodule dry weight, pod number, grain yield, microbial population and phosphorous content in grain whereas the highest stover yield, potassium content in grain and phosphorous content in stover is accumulated where 100% RDF was applied, but that nitrogen content in grain and stover and potassium content in stover were not significantly affected. According to the performance of the crop and the analysis of rhizosphere soil, it can be concluded that the most effective response and good results over the control was found in application of bio-stimulant at 1 L acre$^{-1}$ along with 50% RDF (25:50:30 kg ha$^{-1}$ N:P:K).



**Keywords:** Bio-stimulant, Plant probiotics, Chickpea, Microbial activity




## Introduction

An important part of Indian agriculture is the production of pulses. Chickpea (*Cicer arietinum* L.) is a member of the leguminaceae family. In Ethiopia,Turkey, Burma, Pakistan, Australia, and India, it is extensively grown. Also known as Bengal gram, it is a significant pulse crop for the *rabi* season. According to Pooniya et al. (2015), India makes up roughly 25% of the world's total production of pulses. 21.1% protein, 61.5% carbs, and 4.5% fat are included in chickpeas. It also has high levels of niacin, iron, and calcium. It is fed to animals as well as utilized for human consumption. Modern agriculture faces serious issues such falling soil fertility and pollution contamination of soil and water (Schwarz et al., 2010). There is a need for crop cultivation in unfavourable environments in the context of global climate change and food security, as well as for the sustainable use of precious and limited natural resources through the protection of biodiversity (Szparaga et al., 2019, Postel, 2000 and Del Buono, 2021). Over the years, numerous agricultural systems have proposed the use of biostimulants as a novel and sustainable approach to crop development, particularly in the face of biotic and abiotic stressors (Bertrand et al., 2021, Del Buono, 2021, and Bulgari et al., 2014). According to Bulgari et al. (2014) and Halpern et al. (2015), applying biostimulants is a practical and sustainable way to supplement crop nutrition and may help address environmental problems caused by overfertilization. Du Jardin (2015) defines a bio-stimulant as "any substance or microorganism that applied to plants, regardless of its nutrients content, is able to enhance nutrition efficiency and also abiotic stress tolerance and quality traits" (Ronga et al., 2019). Biostimulants may advance plant development both explicitly and implicitly. Bio-fertilization, sense of root development, resistance to established stressors, and rhizoremediation are a few examples of direct impacts on plant development advancement (Massa et al., 2018, Lugtenberg and Kamilova, 2009 and De Vries et al., 2020). Enhancing plant enzymatic mobility and managing plant microorganisms may indirectly promote plant development (Pérez-Montaño et al., 2014, )Furthermore, bio-stimulatory compounds are known to be a successful way to repair semi-arid areas and damaged ecosystems by boosting microbial activity and improving soil biology (Askari-Khorasgani et al., 2019, Karapouloutidou and Gasparatos, 2019, Kumar et al., 2023, Calvo et al., 2014).

Numerous studies have looked at a variety of stimulants. Ascophyllum nodosum extracts are among the most widely studied biostimulants; they have different effects on different crops and nutritional quality (Pereira et al., 2019, Fan et al., 2013, and Rouphael et al., 2018). They also lessen the effects of water stress on common beans (Petropoulos et al., 2020 and Galvão et al., 2019). Thus, the goal of the current studies is to examine how biostimulants affect the growth, yield, and microbial activity of chickpea (*Cicer arietinum* L.), which are grown in field experiments.

## Materials and Methods

The field trials were conducted during the *rabi* season (December–April) of 2022–23 atthe Instructional Cum Research Farm, College of Agriculture, IGKV, Raipur, Chhattisgarh, India, which is situated at an altitude of 298.58 m above the mean sea level (MSL) at 21°16 N latitude and 81°36 E longitude.Vertisol soil with an alkaline (7.9) soil reaction, low organic carbon (0.54 dS m-1), low available nitrogen (188.23 kg ha-1), medium phosphorous (24.19 kg ha-1), high potassium (505.90 kg ha-1), and low EC were the characteristics of the trial plots' soil. The seed of chickpea, variety RVG-202 was collected from college of Agriculture, IGKV Raipur, (C.G.). Healthy seeds were sown out manually on December 22, 2023, at a depth of 5 cm with an 80 kg $ha^{-1}$ seed rate at a spacing of 45 cm x 10 cm accommodating 22 plants / $m^2$ (5×5 $m^2$ plot size). The experiment was laid out in randomized block design with seven treatments and three replications. The treatments include $T_1$ (Control (without RDF)), $T_2$ (100% RDF), $T_3$ (1 L biostimulant + 200 L water $acre^{-1}$), $T_4$ (1 L biostimulant + 100 L water $acre^{-1}$), $T_5$ (1 L biostimulant + 300 L water $acre^{-1}$), $T_6$ (1 L biostimulant + 200 L water $acre^{-1}$ + 100% RDF) and $T_7$ (1 L Biostimulant + 200 L water $acre^{-1}$ + 50% RDF). Urea, single super phosphate, and murate of potash murate were used to apply the prescribed dosage of fertilizer, which is 25:50:30 kg NPK ha-1, depending on the treatment. At the time of sowing, the full doses of potassium, phosphorus, and half dose of nitrogen were applied while the remaining half of the nitrogen was applied as a top dressing in split doses at 30, 45, and 60 days after sowing. Bio-stimulant was applied by drenching method in root zone. Bio-stimulant was applied three different times: the first application of bio-stimulant was at 20 days after sowing, the second was at 20 days after the first application, and the third at 20 days after the second application through a knapsack sprayer.

The plant received the proper irrigation for its maximum growth and development, and all essential plant protection techniques and intercultural protocols were followed. Observations on five randomly selected plants in each treatment were tagged properly for recording various observations viz. height of plant (cm), dry weight of shoot (g), number of nodule ($plant^{-1}$), nodule dry weight (g) , number of pod ($plant^{-1}$), grain and stover yield (q $ha^{-1}$). N content (%) in grain and stover was determined by Micro-Kjeldahl method as described by Jackson (1973) using auto digestion and distillation Unit. 0.5 gram sample (grain and stover) was taken in digestion tube and digested sample was taken for distillation. The solution collected in the conical flask was titrated by using 0.5 N of H2SO4. The titration value was noted and percentage of nitrogen was calculated and expressed in percentage. Bacterial population in rhizosphere soil was analyzed at 45 and 60 days after sowing by serial dilution plating method as describe by Subba Rao (1982).Nutrient agar medium was utilized to isolate all of the bacteria (CLARK, 1965). Each plot soil sample was plated in triplicate, and the mean values for each sample were calculated. Each set of plating included a Control, which was used to monitor the colony-forming unit. Using detergent powder and distilled and tap water, all of the glassware used in this experiment was cleaned. The dried glassware was sterilized for two hours at 160°C in a hot air oven. Before use, the inoculation needle was heated over a spirit lamp flame after being dipped in alcohol. The media was sterilized by autoclaving at 15 lb pressure for 20 m, and laminar air flow was used for all soil sample isolation and inoculation procedures. For accurate interpretation, every observation made during this experimental study was methodically tabulated. ANOVA was used to statistically analyze the observations, and according to Panse and Sukhatme (1967), a p value of less than 0.05 was deemed statistically significant. By taking the mean value of the observed data, statistical analysis was carried out.

**Results and Discussion**

The data on average plant height were recorded at five different stages (30, 45, 60, 75 DAS and at harvest), which are presented in Table 1. Observation data indicate that the plant's growth was faster up to 30-60 DAS and then a slower growth rate was observed up to 75 DAS at harvest. The growth performance of the crop for the treatment T2 (100% RDF) exhibited the maximum height at 30 and 45 DAS (19.23 and 30.15 cm). All other treatments have also shown equal effect on plant height similar to 100% RDF treatment except control where no significant changes in plant height was observed. The observations at 60 and 75 DAS revealed that maximum height (41.81 and 43.13 cm) was exhibited by the treatment T7 (1:200 dilution with 50% RDF), all other treatments were found to be better than the control in increasing the plant height significantly. At harvest no significant differences were noticed among treatments may be due to senescence. Thus, it was concluded that the maximum plant height was obtained due to application of biostimulant with 50% RDF at the later phase of plant growth. This increases due to  increase in nutrient availability by biostimulant. Similar result of these finding was also supported by Sharanya *et al.* (2022), Gabilondo*et al*. (2023), Fedeli *et. al* (2023), Baradhan *et al. (*2019). The results on shoot dry matter accumulation grew linearly as a crop moved through its development phases. The shoot dry weight of chickpea plant is a good indicator of plant growth which also determines the crop yield. Shoot dry matter of chickpea was quantified at two different stages of crop growth (45 and 60 DAS), which are presented in Table 2. At 45 DAS only the treatment T5 (bio-stimulant @ 1:300 dilutions) was found responsive to increase the shoot dry weight (3.87 g plant$^{-1}$)over control. However, at 60 DAS all the treatments taken under study found efficient to increase the shoot dry weight over control. Maximum dry weight (9.76 g plant$^{-1}$)was attributed to treatment T7 where bio-stimulant was applied @ 1:200 ratio with 50% RDF. Dry weight of shoot was increase due to availability of available soil enriched lignolytic, cellulolytic, and nitrate ammonification. Similar results were obtained by Mukherjee *et al.* (2022). Nodule count of chickpea is presented in table 2. Nodule count was recorded at two different stages of crop growth (45 and 60 DAS). Nodule number was affected by different dilution ratio of bio- stimulant and their use with chemical fertilizers. Results revealed that bio-stimulant significantly affected the number of nodules over control. Maximum number (33.33 and 36.00 g plant$^{-1}$)of nodules was found in treatment T7 in both the growth stages (45 and 60 DAS) respectively, which received 1:200 diluted bio-stimulants with 50% RDF, followed by treatment T6 which received the same diluted bio-stimulant with 100% RDF. This finding was also supported by Sharanya *et al.* (2022). The observations of nodule dry weight were recorded in 45 and 60 DAS, which is presented in table 2. The result of the nodule dry biomass study revealed that all the treatments had shown their effect on nodule dry weight at 45 DAS, except treatment T3 where bio-stimulant was applied @ 1:200 dilution. At 45 DAS Maximum dry weight (0.050 g plant$^{-1}$)of nodule was associated with the application of bio-stimulant @ 1:300 dilution. At 60 DAS all the treatments showed their superiority to increase the nodule dry biomass over control. Maximum dry weight (0.057 g plant$^{-1}$)of nodules was recorded due to application of bio-stimulation with 1:200 dilution with 50% RDF which was found at par with 100% RDF recorded under treatment T2. Similar finding number of nodule and dry weight of nodule was also reported by Garcia *et al. (*2018), Gabilondo*et al.* (2023), Lopez *et al.* (2021). The results revealed that all the bio-stimulant treatments, independent application of chemical fertilizers and their combinations significantly increased the number of pods (Table 2) in chickpea recorded at 60 and 75 DAS. At 60 DAS the treatment T7 (Bio-stimulant at 1:200 dilution + 50% RDF) and T5 (Bio-stimulant at 1: 300 dilution) both produced the highest (28.83) number of pods. At 75 DAS the treatment T7 produced the maximum number of pods (30.00) followed by treatment T5 which produced 29.33 and at par with treatment T7. The result was supported by Praveen *et al.* (2022) and Gabilondo*et al*. (2023). The grain yield of chickpea was significantly increased by use of bio- stimulants and chemical fertilizers applied alone and in different combinations (Table 2 ). Highest grain yield (779.33 kg $ha^{-1}$)) was attributed to treatment T7 where bio-stimulant was applied at 1:200 dilution with 50% RDF. This highest yield was followed by the yield (732.92 kg $ha^{-1}$) obtained in treatment T5 where bio-stimulant was applied at 1:300 dilution. In case of stover yield two treatments $T_2$ and $T_7$ was found significantly superior over control. Highest stover yield (1342.27 kg $ha^{-1}$) was recorded in treatment $T_2$ where 100% RDF was applied, followed by treatment T7 (1087.33 kg $ha^{-1}$) where 50% RDF was applied along with bio-stimulants at 1:200 dilution level. Similar findings was also observed by Dhegavath*et al.* (2022). Population of bacteria in rhizosphere soil was analysed at 45 and 60 days after sowing. Data obtained (Table No. 1) thus revealed that the highest population of total bacteria (25.23 and 26.07 x $10^7$ $g^{-1}$ soil respectively,) was found significantly in treatment $T_7$ due to the application bio-stimulant at 1:200 dilution along with 50% RDF whereas the lowest population of total bacteria at 45 and 60 DAS (17.03 and 17.37 x $10^7$ $g^{-1}$ soil respectively,) was found from treatment $T_1$ (Control, without RDF). The present findings are supported with the result of Tejada et al. (2011), who mentioned that the soil amended with bio-stimulant had the highest soil enzymatic activities and bacterial biomass. Additionally, the use of bio stimulants improved the biological characteristics of the soil and encouraged the growth of vegetation that will shield the soil from erosion and aid in its restoration. Similar to this, Sani et al. (2020) reported that the application of bio stimulants based on *Trichoderma* and bio stimulants extracted from seaweed increased soil fertility and nutrient availability as a result of an abundance of bacterial populations in the rhizosphere. Collectively, findings indicated that biostimulant can be used to activate the beneficial soil and plant-associated microbiota without significant changes in the relative abundance of populations of pathogenic microbial species. Nitrogen content in grain not affected significantly by bio- stimulants application (Table 3). In case of phosphorus content, highest phosphorus content was associated with treatment $T_7$ (Bio-stimulants at 1:200 dilution with 50% RDF) with a mean value of 0.58 %, followed by treatment $T_2$ (RDF) with a mean value of 0.56% Similar findings was reported by Dhegavath*et al*. (2022), Varma *et al.* (2022). Potassium content in grain varied among treatments. However, treatment $T_2$ (100% RDF) accumulated highest K in grain with a mean value of 0.60%, which was only treatment found ignificantly superior over control. Similar findings was reported by Dhegavath*et al*. (2022).Nitrogen and potassium content in stover did not differ significantly by the application of bio- stimulants, as well as by chemical fertilization. However, phosphorus content was varied significantly in stover by imposing treatments which was reflected in the improvement of P-content in stover. Highest phosphorous content (0.28%) was found in stover which was attributed with 100% RDF application in treatment $T_2$. A similar finding was also reported by Rafique *et al*. (2021).

## CONCLUSION

The study found that applying biostimulant with 50% RDF significantly improved plant height, shoot dry weight, nodule number, pod number, grain yield, microbial population, and phosphorous content in grain. However, 100% RDF resulted in higher stover yield, potassium content in grain and phosphorous content in stover, while nitrogen content in grain and stover and potassium content in stover remained unaffected. Further, it can be concluded that the most effective response and good results over the control was found in application of bio-stimulant @ 1 L acre$^{-1}$ along with 50% RDF (25:50:30 kg ha$^{-1}$ N:P:K).

**Table: 1. Effect of Bio-stimulant on plant height of chickpea and the microbial population of chickpea grown soil.**

| Treatment details | Plant height (cm) | | | | | Total bacteria ($10^7$ g$^{-1}$ soil) | |
|---|---|---|---|---|---|---|---|
| | 30 DAS | 45 DAS | 60 DAS | 75 DAS | At harvest | 45 DAS | 60 DAS |
| $T_1$ | 15.55 | 26.34 | 37.20 | 38.15 | 42.72 | 45.33 | 101.67 |
| $T_2$ | 19.23 | 30.15 | 40.64 | 42.05 | 44.02 | 45.33 | 108.33 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| $T_3$ | 18.25 | 28.70 | 40.47 | 40.95 | 43.65 | 43.33 | 111.67 |
| $T_4$ | 18.36 | 28.56 | 39.59 | 40.98 | 43.66 | 54.67 | 104.67 |
| $T_5$ | 18.20 | 28.13 | 39.93 | 41.05 | 43.68 | 52.67 | 112.67 |
| $T_6$ | 18.67 | 28.92 | 39.99 | 40.69 | 43.56 | 54.33 | 118.67 |
| $T_7$ | 18.38 | 28.30 | 41.81 | 43.13 | 44.38 | 47.33 | 88.00 |
| SEm$\pm$ | **0.60** | **1.16** | **1.53** | **1.36** | **1.35** | 2.13 | 3.25 |
| CD ($p$=0.05) | **1.62** | **3.13** | **4.00** | **3.73** | **N.S.** | NS | 10.01 |

## Table: 2. Effect of Bio-stimulant on growth parameters and yield of chickpea.

| Treatment details | Shoot dry weight (gplant$^{-1}$) | | Nodule number (gplant$^{-1}$) | | Nodule dry weight (gplant$^{-1}$) | | Pod number (gplant$^{-1}$) | | Grain yield (kg/ha) | Stover yield (kg/ha) |
|---|---|---|---|---|---|---|---|---|---|---|
| | 45 DAS | 60 DAS | 45 DAS | 60 DAS | 45 DAS | 60 DAS | 60 DAS | 75 DAS | At harvest | At harvest |
| $T_1$ | 3.27 | 5.43 | 12.33 | 15.67 | 0.030 | 0.028 | 21.50 | 21.05 | 528.13 | 835.53 |
| $T_2$ | 3.66 | 9.16 | 30.00 | 32.33 | 0.042 | 0.053 | 25.17 | 26.67 | 604.00 | 1342.27 |
| $T_3$ | 3.21 | 8.00 | 30.33 | 33.33 | 0.027 | 0.045 | 25.83 | 26.00 | 619.20 | 859.07 |
| $T_4$ | 3.35 | 8.33 | 28.33 | 30.67 | 0.042 | 0.042 | 27.83 | 29.00 | 700.00 | 800.00 |
| $T_5$ | 3.87 | 8.50 | 30.67 | 33.67 | 0.050 | 0.047 | 28.83 | 29.33 | 732.92 | 867.07 |
| $T_6$ | 3.18 | 8.32 | 31.67 | 34.00 | 0.045 | 0.047 | 25.33 | 28.67 | 666.27 | 800.40 |
| $T_7$ | 3.52 | 9.76 | 33.33 | 36.00 | 0.049 | 0.057 | 28.83 | 30.00 | 779.33 | 1087.33 |
| SEm$\pm$ | **0.15** | **0.38** | 1.31 | 1.47 | 0.002 | 0,003 | 1.11 | 1.03 | 25.87 | 29.73 |
| CD ($p$=0.05) | **0.43** | **1.04** | **3.48** | **3.88** | **0.005** | **0.007** | **2.96** | **2.75** | **67.63** | **103.63** |

## Table 3. Effect of bio-stimulants on nutrient content in grain and stover of chickpea.

| Treatment details | Nutrient content in grain (%) | | | Nutrient content in stover (%) | | |
|---|---|---|---|---|---|---|
| | **N** | **P** | **K** | **N** | **P** | **K** |
| $T_1$ | 3.12 | 0.43 | 0.52 | 0.69 | 0.24 | 1.24 |
| $T_2$ | 3.24 | 0.56 | 0.60 | 0.71 | 0.28 | 1.32 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $T_3$ | 3.15 | 0.54 | 0.55 | 0.72 | 0.27 | 1.27 |
| $T_4$ | 3.19 | 0.52 | 0.56 | 0.68 | 0.26 | 1.30 |
| $T_5$ | 3.2 | 0.54 | 0.55 | 0.63 | 0.27 | 1.27 |
| $T_6$ | 3.21 | 0.45 | 0.56 | 0.66 | 0.25 | 1.26 |
| $T_7$ | 3.21 | 0.58 | 0.56 | 0.68 | 0.27 | 1.23 |
| SEm± | 0.09 | 0.02 | 0.02 | 0.02 | 0.01 | 0.04 |
| CD ($p$=0.05) | **NS** | **0.05** | **0.05** | **NS** | **0.02** | **NS** |



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